

SPECTRAL SENSITIVITY OF THE CHAETOGNATH *SAGITTA HISPIDA* CONANT

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ABSTRACT

Using phototaxis as a behavioral measure of photosensitivity, the spectral sensitivity of *Sagitta hispida* Conant (Chaetognatha) was determined. *S. hispida* is most sensitive to blue-green light, with maximum sensitivity at 500 nm. From 400-580 nm, the shape of the action spectrum for phototaxis approximates an absorbance spectrum for a rhodospin-based visual pigment. This suggests that photoreception is mediated largely by a single major pigment. An accessory pigment may play a role in photoreception at longer wavelengths. *S. hispida* is adapted for greatest photosensitivity wherever blue-green light dominates the available spectrum. This finding is consistent with the geographical range of this species, which comprises relatively clear blue-green tropical and subtropical seas.

INTRODUCTION

At Beaufort, North Carolina, we determined through field studies that the chaetognath *Sagitta hispida* Conant performs a nocturnal diel vertical migration. For adult animals, the migration is characterized by near absence from the 7 m water column by day, and appearance at all depths shortly after sunset (Sweatt and Forward, 1985). It is generally believed that, in performing such vertical migrations, zooplankton are responding to some aspect of changes in light intensity associated with sunset or sunrise (Forward, 1976). It was hypothesized that the vertical migration of *S. hispida* depends on photoreception for its initiation. Accordingly, laboratory studies were undertaken to examine the role that light might play in determining the timing of the ascent phase of the migration.

Initial investigations dealt with the basic photophysiology of *S. hispida*, and we report here our findings concerning the spectral sensitivity of this species. The spectral dependence of variations in phototactic tendency was taken as a measure of photosensitivity. It was found that *S. hispida* is most sensitive to blue-green light. Photoreception appears to be mediated by a rhodopsin-like visual pigment with maximal absorbance near 500 nm. This finding is discussed with reference to the spectral distribution of light in this chaetognath's environment.

MATERIALS AND METHODS

Chaetognaths were collected in plankton nets suspended in the tidal flow beneath the Piver's Island Bridge at Beaufort, North Carolina. Net mouth diameters ranged from 0.25 to 1.0 m, and all nets were constructed of 0.500 mm Nytex® mesh, which

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retains chaetognaths > 6 mm in length. To minimize damage to the chaetognaths, a large cod end receptacle (4 l capacity) was attached to each net. Collections were made at night, when light sources consisted of distant street lamps and a small red light on the bridge platform. The animals were transported to the laboratory in darkness and not exposed to bright light until the following morning. The day after capture, *S. hispidus* were separated from other zooplankton with the aid of large bore pipettes and a dissection microscope.

In the laboratory, the chaetognaths were kept in aerated 30 l glass aquaria filled with sea water filtered to remove particles larger than 5 μm . Sea water was obtained from the Duke University Marine Laboratory's running sea water system, and its temperature and salinity (19–22°C, 34–36 ppt) were close to the field values at the time of collection (13.5–20.0°C, 33–35 ppt). During experiments, temperature and salinity changes were minimized to avoid eliciting photoresponses or other behaviors which might be related to escape from stressful environments (e.g., positive phototaxis upon exposure to a salinity increase; Forward, 1976).

The aquaria were maintained under a 12L:12D photoperiod (cool white fluorescent lights; light intensity: 10^{19} photons $\text{m}^{-2} \cdot \text{s}^{-1}$, measured at the tops of the aquaria). The chaetognaths were supplied daily with food organisms (newly hatched *Artemia salina* nauplii), and placed in new, filtered sea water at least every other day.

Prior to each phototaxis experiment, groups of 20–25 *S. hispidus* were placed in sea water-filled 50 ml beakers. The animals were dark adapted for at least 1.5 h before each experiment. To avoid possible complications in interpretation of results due to endogenous rhythms in activity or photosensitivity, the experiments were performed from 1330 to 1830 h each day.

The experimental light source was a slide projector (Spindler and Sauppe, Model SL-750), equipped with a 300 or 750 W incandescent bulb. Heat was removed from the light with heat filters (Corning, #1-75), and hot mirrors (Baird Atomic, Inc.), while wavelength was controlled by interference filters (6.8–11.5 nm half band width; Ditic Optics, Inc.). Light intensity was regulated with neutral density filters (Ditic Optics, Inc.). The projector was housed in a box such that the projected light exited only from a small aperture.

The test vessel was a horizontal trough, 41 × 8 × 7 cm, constructed of transparent plastic (Lucite®). The long axis of the trough was aligned with the optical axis of the projector. Along its length, the vessel was divided by partitions into five equivalent sections. The partitions were attached to a horizontal cross-piece, and could be moved vertically in unison. Light intensity was measured using a laboratory photometer (EG & G, Model 550). The photometer probe was placed inside the empty vessel, against the end closest to the light source, for measurement.

In performing a test, the vessel was filled with sea water, and the partitions put in place. In darkness, a beaker of dark-adapted *S. hispidus* was then gently immersed in the center section of the trough, rotated to release the chaetognaths, and removed. After pausing for 30 s in darkness to allow the animals to adjust to the chamber, the partitions were gently withdrawn and the light source switched on. Following a three-minute stimulus period, the partitions were replaced, and the distribution of animals among the sections of the vessel was determined. Control experiments were conducted in the same manner, except that the animals were not irradiated. Chaetognaths found in the section of the test chamber closest to the light source were considered positively phototactic, in that they swam at least 8 cm toward the light source. Those in the distal section of the chamber were considered negatively phototactic. For each test performed, the percent of animals exhibiting positive or negative phototaxis was determined. The three-minute stimulus period was chosen so that over the range of

stimulus strengths employed, the animals would exhibit both saturated and control level phototactic responses.

The stimulus-response function for positive phototaxis was determined at 15 wavelengths spaced at 20 nm intervals over the region 400–680 nm. The data were used to determine an action spectrum for phototaxis. Details of the calculation of the action spectrum are given with the results.

RESULTS

S. hispida displayed only positive phototaxis in these experiments. Negative phototaxis rarely exceeded the 10% level, with a mean negative control response of 7.2% (SEM: 0.7). The swimming pattern during phototaxis was the characteristic dart-and-sink motion described for *S. hispida* by Feigenbaum and Reeve (1977), and for *S. crassa* by Goto and Yoshida (1981, 1983). Quick target-aiming behavior, a type of light adapted startle response described by Goto and Yoshida (1981), was not observed in these experiments, but has been seen in tests with light adapted *S. hispida* (Sweatt, 1983).

As data were accumulated, plots of percent phototaxis *versus* stimulus intensity indicated that a roughly hyperbolic stimulus-response relationship held for positive phototaxis. Over the lower part of the range of stimulus intensities employed, responses at each wavelength were generally below 10%. This level of responsiveness was little different from that seen in dark control experiments (Mean: 3.9% SEM: 0.5). As stimulus intensity was increased at each wavelength, responses rose sharply between 10% and 50% phototaxis, and leveled off at about 50%. In order to accurately characterize the relationship between stimulus intensity and response strength, subsequent tests were performed at stimulus intensities in the range which elicited responses lying in the rising portion of the hyperbola (*i.e.*, 10–50% phototaxis). Figure 1 shows, for

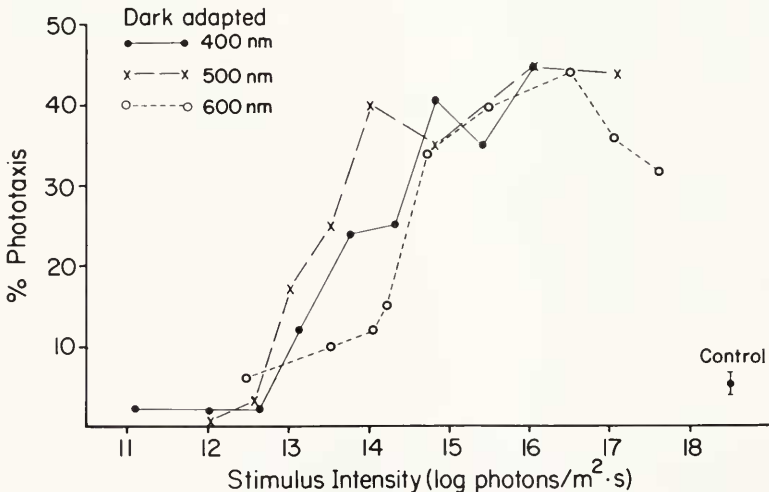


FIGURE 1. Representative stimulus-response functions for dark adapted positive phototaxis by *S. hispida*. % Phototaxis is the proportion of animals swimming a distance of at least 8 cm toward a collimated light source within a three minute period. Stimulus light intensity is expressed in log units. Each point represents the mean of three tests. For clarity, standard errors were omitted from the plot. Control responses were determined by performing phototaxis tests in darkness. Control level and standard error based on 60 tests.

three representative wavelengths, stimulus-response functions for responses lying below 50% phototaxis.

The spectral dependence of the phototactic response can be displayed by an action spectrum. The action spectrum is determined by calculation of the quantal flux necessary at each wavelength to elicit a response of a given magnitude. This method provides a measure of spectral dependence which depends only on the number of quanta absorbed by the system under study, and is not affected by the choice of response (Rodieck, 1973). Thus, it is possible to compare a behaviorally determined action spectrum with, for example, an absorbance spectrum for a photopigment.

To determine the action spectrum for phototaxis by *S. hispidus*, the percent response data were subjected to arcsine transformation (Sokal and Rohlf, 1981) and a linear regression was fitted to the points lying in the steeply rising portion of the stimulus-response function for each test wavelength (*i.e.*, for all responses between 10% and 50% phototaxis). The regression technique provided a method for objectively describing and comparing the stimulus-response functions. Analysis of covariance (Snedecor and Cochran, 1967) revealed that the slopes of the 15 regression lines were not significantly different from each other, indicating that the shape of the stimulus-response function was essentially the same at all test wavelengths. Regression line intercepts differed significantly ($P < .01$), an indication of spectral variation in the sensitivity of the phototactic response. The 30% phototaxis response was chosen as the criterion response for the action spectrum, as this value lies at the midpoint of the response range used to fit each linear regression. For each test wavelength, the stimulus intensity necessary to elicit a 30% phototactic response was estimated from the appropriate regression equation. The reciprocal of this quantity was then plotted, on a relative scale, against wavelength (Fig. 2).

The action spectrum shows that, based on phototactic responsiveness, *S. hispidus* is most sensitive to blue-green light, with maximum sensitivity at 500 nm. Sensitivity at wavelengths above 620 nm was an order of magnitude lower than the lowest sensitivity shown in Figure 2. Included in Figure 2 is an absorbance spectrum for visual pigment having maximum absorbance at 500 nm. This curve was calculated from a nomogram based on the characteristic shapes of absorbance spectra for rhodopsin-based visual pigments (Dartnall, 1953; Ebrey and Honig, 1977). The absorbance spectrum approximates the action spectrum from 400–520 nm, but deviates from it at longer wavelengths.

DISCUSSION

Positive phototaxis has been reported for chaetognaths by Esterly (1919) and Pearre (1973). Esterly's observations were basically anecdotal, in that no dark control experiments were performed, and the animals (*Sagitta eunertica*; Alvarino, 1965) could initially swim only toward the light sources. In work with *Sagitta elegans*, Pearre (1973) included proper controls, and allowed animals to swim either toward or away from a light source. He reported that 58.9% of the animals swam toward the light, while 44.7% swam away from the light in a horizontal tank. The apparent weakness of the positive phototactic response may have been due to use of a stimulus period of 20 minutes, which may have obscured initially strong phototactic responses. The unequivocal positive phototaxis reported here for *S. hispidus* appears to be comparable to that described for dark adapted *Sagitta crassa* by Goto and Yoshida (1981, 1983). Both of these species were tested using relatively short stimulus periods (3 minutes and less than 10 minutes, respectively).

S. hispidus's response was useful as a measure of photosensitivity, and allowed

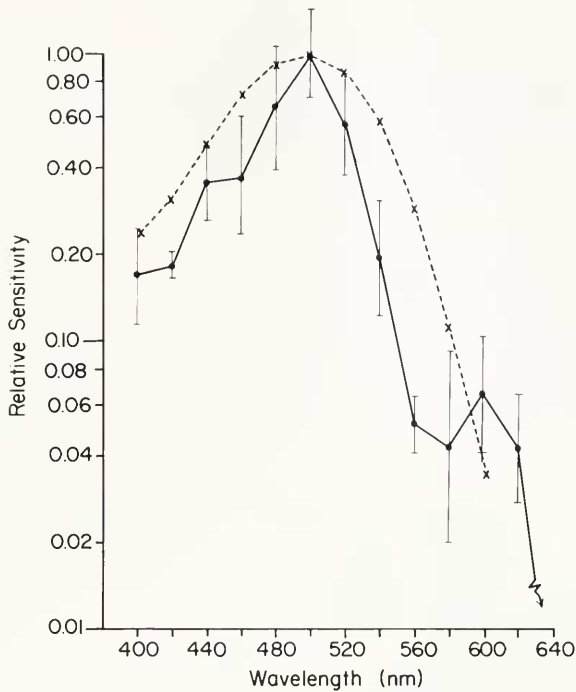


FIGURE 2. Action spectrum for positive phototaxis by *S. hispidia*. For each wavelength the reciprocal of the quantal flux required to elicit 30% phototaxis was calculated. Each value was then divided by the value obtained at 500 nm, where sensitivity was maximal. This quotient is shown as the relative sensitivity. Error bars were calculated similarly, based on the standard error of the 30% phototactic response at each wavelength. Dashed line shows an absorbance spectrum calculated from a nomogram for a visual pigment having maximum absorbance at 500 nm.

the construction of an action spectrum for phototaxis (Fig. 2). The action spectrum shows that *S. hispidia* is most sensitive to blue-green light, with maximum sensitivity at 500 nm. In Figure 2, the shape of the action spectrum corresponds roughly with the absorbance spectrum of a rhodopsin-based visual pigment with maximum absorbance at 500 nm (Ebrey and Honig, 1977). This may be interpreted as evidence that *S. hispidia* possesses a visual pigment which has an absorbance maximum near 500 nm. The small shoulder in the action spectrum at 600–620 nm could indicate the presence of another visual pigment, with an absorbance maximum near 600 nm.

Experimental evidence suggests that chaetognaths are not visual predators (Reeve, 1964; Feigenbaum and Reeve, 1977). It is probable that chaetognath photoreception is mainly concerned with light dependent orientation behaviors, which could include vertical migration. Among zooplankton, light intensity is considered the most important environmental cue involved in vertical migration (Forward, 1976). If chaetognath photoreceptors are primarily used during vertical migration, then it could be expected that their spectral sensitivity should match the spectrum of available light in their underwater environment. Alternatively, a spectral sensitivity maximum which is offset from the environmental spectral transmission maximum may signify an adaptation to enhance contrast sensitivity, which might be useful in object recognition (Lythgoe, 1966; Forward and Cronin, 1979).

In the open ocean, the median of the photon spectral transmission function lies at 470 nm (McFarland and Munz, 1975). Accordingly, open ocean zooplankton which undergo vertical migration frequently have visual pigments with main absorption maxima in the region 460–495 nm (Forward, 1976). Inshore, higher concentrations of phytoplankton, detritus, and complex organic molecules shift the spectral transmission maximum to longer wavelengths (>500 nm). For example, in the estuary where *S. hispidus* was collected, the photon transmission maximum lies at 575 nm (Sweatt, 1983). The absorbance maxima of the visual pigments of many coastal and estuarine zooplankton are in the region 500–600 nm (e.g., Stearns and Forward, 1984). *S. hispidus*, with maximum photosensitivity at 500 nm, could be considered to be better adapted to open ocean spectral environments than to estuaries.

The geographical range of *S. hispidus* comprises the tropical and subtropical eastern Atlantic (Alvarino, 1965). Throughout most of this region, but especially offshore, this species is more likely to encounter clear blue water than the greenish yellow waters characteristic of temperate areas (Smith, 1974). However, the shape of the action spectrum for phototaxis by *S. hispidus* suggests the presence of a second visual pigment, with maximum absorbance near 600 nm. Such an accessory pigment may provide for an increase in photosensitivity in estuarine waters, where much of the available light lies at longer wavelengths.

Thus the spectral sensitivity of *S. hispidus* seems to be adapted to available light. This agreement suggests that vision could be involved in vertical migration. The roles of phototaxis and vertically oriented swimming in the diel vertical migration of *S. hispidus* are addressed in a separate publication (Sweatt and Forward, 1985).

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LITERATURE CITED

- ALVARINO, A. 1965. Chaetognaths. *Oceanogr. Mar. Biol. Ann. Rev.* **3**: 115–194.
- DARTNALL, H. J. A. 1953. The interpretation of spectral sensitivity curves. *Brit. Med. Bull.* **9**: 24–30.
- EBREY, T. G., AND B. HONIG. 1977. New wavelength dependent visual pigment nomograms. *Vision Res.* **17**: 147–151.
- ESTERLY, C. O. 1919. Reactions of various plankton animals with reference to their diurnal vertical migrations. *Univ. Calif. Publ. Zool.* **19**: 1–83.
- FEIGENBAUM, D. L., AND M. R. REEVE. 1977. Prey detection in the Chaetognatha: response to a vibrating probe and experimental determination of attack distance in large aquaria. *Limnol. Oceanogr.* **22**: 1052–1058.
- FORWARD, R. B., JR. 1976. Light and diurnal vertical migration: photobehavior and photophysiology of plankton. *Photochem. Photobiol. Rev.* **1**: 157–209.
- FORWARD, R. B., JR., AND T. W. CRONIN. 1979. Spectral sensitivity of larvae from intertidal crustaceans. *J. Comp. Physiol.* **133**: 311–315.
- GOTO, T., AND M. YOSHIDA. 1981. Oriented light reactions of the arrow worm *Sagitta crassa* Tokioka. *Biol. Bull.* **160**: 419–430.
- GOTO, T., AND M. YOSHIDA. 1983. The role of the eyes and CNS components in phototaxis of the arrow worm *Sagitta crassa* Tokioka. *Biol. Bull.* **164**: 82–92.
- LYTHGOE, J. N. 1966. Visual pigments and underwater vision. Pp. 375–391 in *Light As An Ecological Factor*, R. Bainbridge, G. C. Evans, and O. Rackham, eds. Blackwell, Oxford.
- McFARLAND, W. N., AND F. W. MUNZ. 1975. Part II. The photic environment of clear tropical seas during the day. *Vision Res.* **15**: 1063–1070.

- PEARRE, S. JR. 1973. Vertical migration and feeding in *Sagitta elegans* Verrill. *Ecology* **54**: 300-314.
- REEVE, M. R. 1964. Feeding of zooplankton, with special reference to some experiments with *Sagitta*. *Nature* **201**: 211-213.
- RODIECK, R. W. 1973. *The Vertebrate Retina*. Freeman, San Francisco. 1044 pp.
- SMITH, R. C. 1974. Structure of solar radiation in the upper layers of the sea. Pp. 95-119 in *Optical aspects of oceanography*, N. G. Jerlov, ed. Academic Press, New York.
- SNEDECOR, G. W., AND W. G. COCHRAN. 1967. *Statistical Methods*, 6th ed. Iowa State University, Ames, Iowa. 593 pp.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*. 2nd ed. Freeman, San Francisco. 776 pp.
- STEARNS, D. E., AND R. B. FORWARD, JR. 1984. Photosensitivity of the calanoid copepod *Acartia tonsa* Dana. *Mar. Biol.* **82**: 85-89.
- SWEATT, A. J. 1983. Photobiology of the chaetognath *Sagitta hispidata* Conant. Ph. D. Thesis. Duke University, Durham, North Carolina.
- SWEATT, A. J., AND R. B. FORWARD, JR. 1985. Diel vertical migration and photoresponses of the chaetognath *Sagitta hispidata* Conant. *Biol. Bull.* **168**: 18-31.